

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT(S): Kalle SAKSELA et al.
APPLICATION NO.: 09/579,894 GROUP: 1627
FILED: May 26, 2000 EXAMINER: B. Celsa
FOR: METHODS AND MATERIALS FOR GENERATING SH3
DOMAINS WITH TAILORED BINDING PROPERTIES

DECLARATION SUBMITTED UNDER 37 C.F.R. §1.132

Honorable Commissioner of Patents
Washington, D.C. 20231


I, Dr. Marius SUDOL do hereby declare the following.

I am an associate professor in Mt. Sinai School of Medicine, New York, NY. I am a researcher in the field of protein domains and I am an inventor on two USA patents. I obtained my Ph.D. degree at the Rockefeller University in New York City, USA. Dr. Kalle Saksela was a colleague of mine from the tenure at the Rockefeller University.

In the recent past I had a chance to analyze some of the patent proceedings of Dr. Saksela. Based on my analysis I would like to state the following: The capacity of the RRT-SH3 domains revealed in the publication from the Saksela lab (Hiipakka et al., 1999 J. Mol. Biol. 293:1097-1106), the subject matter of which corresponds to the above-identified patent application, is novel and cannot be directly compared to the finding published earlier by the laboratory (Lee et al., 1995, EMBO J. 14:5006-5015). In my judgement the idea of generating artificial, ligand-tailored SH3 domains became obvious only from the publica-

tion of Hiipakka et al., 1999 and not from the previous report of Lee et al. from 1995.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.


Dr. Marius SUDOL


Date

SENT BY MAIL TO:

MaryAnne Armstrong
BIRCH, STEWART, KOLASH & BIRCH, LLP
P.O. Box 747
Falls Church,
VIRGINIA, 22040-0747

An alignment showing approximately half of all SH3 domains found in the human genome. The positions of the five beta-strands of the SH3 protein fold are indicated with "b", and are separated by slashes ("/") corresponding to the loop regions or by a 3_{10} -helix ("3"). The first loop region is known as the RT-loop. The RT-loop consist of a region that is variable in sequence and length (typically six amino acids but ranging among the SH3 domains shown below from 3 to 14) flanked by four and eight relatively well-conserved residues. The variable region of the RT-loop (colored yellow) can be easily identified in the sequence of different SH3 domains based on the adjacent conserved amino acid motifs "ALYDY" and "DL" (colored green). The "ALYDY motif" almost invariably consists of a combination of the amino acids A/V - L - Y/F/W/H - D - Y/F/H, whereas the "DL motif", almost invariably consist of a combination of the amino acids D/E-L/I/V.

The present invention is based on our observation that by replacing the variable region of the Hck-SH3 domain of (EAIHHE; residues 69-74 of Hck protein) with completely random six-amino substitutions artificial SH3 domains (RRT-SH3 domains) can be generated that can bind with unnaturally high affinities to various ligands of natural SH3 domains despite the fact that their engineered RT-loop sequences bear no resemblance to natural SH3 domains. It is important to note, that we have successfully used Hck-derived RRT-SH3 domains to target with high affinity also ligands that (unlike HIV Nef) normally show no binding to Hck-SH3.

Although our Hck-derived RRT-SH3 library (carrying approximately 64 million different variants) appears sufficient for targeting almost any SH3 ligand protein (and thus to develop competitive inhibitors for virtually any SH3 domain), most if not all of the other SH3 domains could also be used as scaffolds for generating similar RRT-SH3 libraries. This could be done by replacing the variable part of the RT-loop (as defined above and colored yellow in the alignment below) by a stretch of random residues. Although this was the case in our Hck-derived RRT-SH3 library, the length of the randomized sequence would not need to be the same as in the original SH3 domain, and could range anywhere between 4 and 20.

ALYDY	DL	
V F F	E I	
W H	V	
H		
bbbbbb-----bbbbbb-----bbbbbb3333bbbbbb		
IIVV ALYDY MEAE-----IH HD ELSFQKGDQMVVLEES-----GEWWKARSL-----ATRKEGYIPSNYVARV		Hck
TLFV ALYDY MEAE-----RT ED ELSFHKGEKFQILNSS-----EGDWWEARSL-----TTGETGYIPSNYVAPV		Fyn
ELVL ALYDY QOE-----KSPREVTMKKGDIITLLNST-NKARHREWLCDWWKVEVN-----DRQGFVPAAYVKKL		alpha-II spectrin
QRVM ALYDY FOA-----RSPREVTMKKGDIITLLSSI-----NKDWWKVEAA-----DHQGI VPAVYVRR		alpha-I spectrin
NLVIAL ALYDY MEP-----SHD GL GFEKGEQLRILEQS-----GEWWKAQSL-----TTGQEGFIPFNFVAKA		Lck
DIVV ALYDY PDG-----IHP DL ELSPFKKGEKMKVLEEH-----GEWWKAKSL-----LTKKEGFIPSNYVAKL		Lyn
HVVO ALYDY PSS-----SNDEELNFEKGDVMDVIEKP-----ENDPEWWKCKRI-----NGMVGVLVPKNYVTVM		Nck1 #3
PQCK ALYDY EEV-----KDKEADKDCLPFAKDDVLTIVIRRV-----DENWAEGLMA-----DKIGIFPISYVEFN		POSH#2
EWCE ALYDY SETA-----ETSD EL SPFKRGDRIQILRL-----DSDWCRGRLQ-----DREGIFPAVFVRPC		SH3d19 #4
MEAV ALYDY ETA-----SGED EL SFHTGDVLKILSNQ-----EEWFKAELG-----SQEGYVVPKNFIDIQ		GADS/Grp2 #1
PHAV ALYDY DEPA-----EQVD EL NLTSGEIVYLLEKI-----DTDWYRGNCR-----NQIGIFPANYVKVI		SH3d19 #2
RIFV ALYDY DPVSMSPNPDAGEEELPFREGQILKVPQDK-----DADGFYQGGEG-----GRTGYIPCNMVAEV		PRAX-1 #2
NTYV ALYDY KFVP-----QEN ED ELMRPGDIITLLEDS-----NEDWWKGIQ-----DRIGFFPANFVQRL		Stac
TI FV ALYDY MEAE-----RT ED ELSPFKKGERFQIINNT-----EGDWWEARSI-----ATGKNGYIPSNYVAPA		Yes
SQVE ALYDY SYEA-----TQPE EL EFQEGDIILVLSKV-----NEEWLEGECK-----GKVGIFPKVFVEDC		p67-phox #2
IEVT ALYDY SEEG-----QQPG EL NFQAGDRITVLSKT-----DSHFDDWEGKLR-----GQTGI FPAANYVTMN		Homology to Grb2
HFVV ALYDY MTA-----MND EL QMLKGEKLQVLKGT-----GDWWLARSL-----VTGREGYVPSNFVARV		Blk
TTFV ALYDY YES-----RT ED ELSPFKKGERLQIVNNT-----RKVDVREGDWLAHSL-----STGQTGYIPSNYVAPS		N-Src
TTFV ALYDY YES-----RT ED ELSPFKKGERLQIVNNT-----EGDWLAHSL-----STGQTGYIPSNYVAPS		Src
HSMV ALYDY NP-QESSPNMDVEAEELPFRAQDVITVFGGM-----DDDGFFYEGELN-----GQRLVPSNFLEGP		PRAX-1 #3
VCAE ALYDY BHVT-----MDD EL QLGFKAGDVIIVMDAT-----NREWWGRVA-----DGEGWFPASFVRLR		Asef / ARHGEF4
IQV ALYDY DELP-----REPC EL NALRAAEYLIILEKY-----NPHWWKARDR-----LGNEGLIPSNYVTEN		Txx
VCAE ALYDY BHVT-----MDD EL QLGFKAGDVIIVLEAS-----NKDWWGRSE-----DKEAWFPASFVRLR		Homology to Asef
VSAE ALYDY BHVT-----MAN EL AFKAGDVIIVLDAS-----NKDWWWGQID-----DEEGWFPASFVRLW		ARHGEF9
GVIY ALYDY MEP-----QND EL ELPMKEGDCMTIIHRE-----DEDEIEWWWARLN-----DKEGYVPRNLLGLY		53BP2
GVAY ALYDY MEAE-----QNS EL ELSFHEGDAITILRRK-----DESETEWWARLG-----DREGYVVPKNLLGLY		ASPP1
GAVY ALYDY MSA-----EFG EL ELSFREGESVTVLRRD-----GPEETDWWAAALH-----QEGYVPRNYFGFL		RAI
PTVV ALYDY MTA-----NRSE EL TIHRGDIIRVFFKD-----NEDWWYGSIG-----KGQEGYFPANHVASE		Homology to Tipd
RKAR ALYDY DA-----ANST EL ELSLADEVITVFSVV-----GMSDSDWLMGERG-----NQKGKVPITYLELL		endophilin B1
RKAR ALYDY MEAE-----ADS EL ELALLADELITVYSLP-----GMDPDWLIGERG-----NKKKGKVPITYLELL		endophilin B2
RKAK ALYDY ERG-----ENE EL ELSPKAGDII TELESV-----DDDWMSGELM-----KSGGIFPKNYIQFL		SH3d19 #5
EEYI ALYDY FPSS-----VEPG EL ELTFTEGEEILVTQKD-----GEWWTGSIG-----DRSGIFPSNYVKPK		Intersectin 2 #3
GIAI ALYDY DECA-----RDM EL ELSLKGDVVVKIYTKM-----SANGWWRGEVN-----GRVGWFPSTYVEED		Vav3 #2

NMFVALLHSYSA	-----HGPDELDLQKGEGRVVLGKC-----	QDGWLRGVSL-----	VTGRVGIFPNNYVIPI
GTAKARMDCA	-----RDRSLSLKEGDI I KILNKK-----	QQQGWWRGEIY-----	GRVGVFPANYVEED
LLARALMNCNP	-----DCSDELAFSRGDILTILEQH-----	VPSEGWKCLLH-----	GRQGLAPANRLQIL
VRVRALMDYDG	-----QEQDELFSKAGDELTKLGEE-----	DEQGWCRGRLD-----	SGQLGLYPANYVEAI
VRVRALMDYEG	-----QEHDLSFKAGDELTKMEDE-----	DEQGWCKGRLD-----	NGQVGLYPANYVEAI
IPIKAIKDMRQ	-----IEMTIYKDDCVLANNS-----	HRKKWKVISPT-----	GNEAMVPSVCFTVP
PRCRALMDYVG	-----QDVDELFSNVNEVEIEIMED-----	PSGWWKGRLLH-----	GQEGLPFGNYVEKI
YTAVALMDYQA	-----AGDDELSPDPDDITITNEMI-----	DDGWWRGVCK-----	GRYGLFPANYVELR
ISAVALMDYQG	-----EGSDELSFDPDDIVTDIEMV-----	DEGWWRGRCH-----	GHFGLFPANYVKLL
NVYLALMAKPK	-----QKSDELHLKHEMYRVLEKC-----	QDGWFKGASL-----	RTGVSGVFPNGYVTPV
TKARVMDFAA	-----EPGNNELTVNAGEIITITNPD-----	VGGGWLEGRNI-----	KGERGLVPTDYVEIL
LRARALMDERS	-----ENPGBSLREHEVLSLCSEQ-----	DIEGWLEGVNS-----	RGDRGLFPASYVQVI
RRMVALMDYDP	-----RESSPNVDVEAEITFTGDIITVFGI-----	DEGGFYYGELN-----	GQKGLVPSNFLEEV
RRAKALLDFER	-----HDDDELGFGRKNDIITIVSQK-----	DEHCWVGELN-----	GLRGWFPKAFVEVL
VEAIMEYDYQA	-----QHDDELITISVGEIITNIRKE-----	DGGWWEQGIN-----	GRRGLFPDNFVREI
VDYIMEYDYDA	-----VHDDELITIRVGEIIRNVKKL-----	QEGWLEGEELN-----	GRRGMFPDNFVKEI
HYFVALMDYQA	-----RTAEDLSFRAGDKLQVLDL-----	HEGWWFARHL-----	EKRDRGSSQQLQGYIPSNYVAED
VLAKALMDNVA	-----ESPDELSPFRKGDIMTVLEQD-----	TQGLDGNWCLSLH-----	GRQGI VPGNRLKIL
LMARALMDNVP	-----ECAEELAFRKGDIITVIEQN-----	TGLEGWCLSLH-----	GRQGI VPGNRVKLL
QLARALMDNTA	-----ESPOELSPFRGDVLRVLORE-----	GAGLDGNWCLSLH-----	QQGI VPGNRVKLL
FSYQALMDYIP	-----QNDDLELRLDGDIVDMMEKC-----	DDGWFVGTSR-----	RTKQFGTFPGNYVKPL
EPFOALMDMTP	-----RNEDELRESDDVIDVMEKC-----	DDGWFVGTSR-----	RTKFFGTTFPGNYVKRL
RTYRALMDYSA	-----QDEDEVSPRDGDIVNVQPI-----	DDGWMYGTVQ-----	RTGRTGMLPANYIEFV
KRYRALMDYSA	-----ADEDEVSPQDGDITVNVQOI-----	DDGWMYGTVE-----	RTGDTGMLPANYVEAI
KI FRAMMDYMA	-----ADADEVSFKDGDIAINVQAI-----	DEGWMYGTVQ-----	RTGRTGMLPANYVEAI
VVARALMDFAA	-----VSEEBISFRAGDMLNLALKE-----	QQPKVRGWLLASLD-----	GQTTGLIPANYVKIL
VYRVALMPEES	-----RSHDEITIQPGDIVMVKGEW-----	VDESQTGEPGWLLGELK-----	GKTGWFPANYAEKI
VYRVALMPEES	-----RSHDEITIQPGDIVMVDESQ-----	TGEPGWLLGELK-----	GKTGWFPANYAEKI
VNYRVALMPEEA	-----RNHDEMSFNSGDI IQVDEKT-----	VGEPGWLYGSFQ-----	GNFGWFPANYVEKM
PVWTALMDYEP	-----SGQDELALRKGDREVLSD-----	AAISGDEGWAGQVG-----	GQVGIFPSNYVSRG
RLCKALMSFOA	-----RQDDELANLEKGDIVIIHEKK-----	EEGWFGSLN-----	GKKGHFPAAYVEEL
FKVOALMDYTA	-----TDTDELQLKAGDVVLVIFPQ-----	NPEEQDEAGLWGVKESDWNQHKLEKCRGVFPENFTERV	
YKVETLHDEFA	-----ANSDELTLQRGDVVLVPSD-----	SEADQDAGLWGVKESDWNQHKLEKCRGVFPENFTERV	
RQCKALMFIPI	-----QNEDELLEKVGDIIDINEEV-----	EEGWWSGLTN-----	NKLGLFSPNFVKEL
QEYRALMDYTA	-----QNPDELDLASGDIILEVILEG-----	EDGWWTVERN-----	GQRGVFPGSYLEKL
PVWTALMDYEA	-----AGDEELTLRRGDRVQVLSQD-----	CAVSGDEGWWTGQLP-----	SGRVGVFPSPNYVAPG
VRVRALMDYAG	-----QEADELSFRAGEELLKMSSE-----	DEQGWCCQLQ-----	SGRI GLYPANYVECV
YQYRALMDYKK	-----EREEDDLHLGDIITVNGSSDGEARPEEIGWLNNGYNE-----	TTGERGDFPGTYVEYI	
FQYRALMFERR	-----ERPDELLELPGDVVLSRAAEGGERCPQSVGMPLGNE-----	RTQRGDFPGTYVEFL	
LKGRALMDYHS	-----ENKEBISIQDDELVI FSET-----	SLDGNWLGQNS-----	RGETGLFPASVVEIV
PQCKALMADA	-----QDTDELSPNANDIIDIIKED-----	PSGWWTGRLLR-----	GKPGLPFNNTVTKI
KKVVALMDYMP	-----MNANDELQLRKGDYFIIIEES-----	NLPWWRRARDK-----	NGQEGYIPSNYVTEA
TVVI ALMDYQT	-----NDPOELALRRNEEYCLLDSS-----	EIHWWRVQDR-----	NGHEGYVPSYLVEK
KYVKILMDYTA	-----RNANDELVLKDEVLVLELDG-----	RQWKLRSR-----	SGQAGYVPCNILEGA
RIFVALMDYDPLTMSPNPDAAEBELPFKEGQIKVYGDK	-----DADGFYRGETC-----	ARLGLIPCNMVSEI	
VVVVALMDYVA	-----QQEQELDIKKNERLWLLDSS-----	KSNWRVRNS-----	MNKTGFVPSNYVERK
VIVIALMDYTA	-----QQDQELDIKKNERLWLLDSS-----	KTWWRVRNA-----	ANRTGYVPSNYVERK
MESVALMSFOA	-----TESDELA FNKGDITLKI LME-----	DDQNWKAEELR-----	GVEGFIPKNYIRVK
LKVRALMDYFN	-----LHDPTALNVRAGDVIITVLEQH-----	PDGRWKGHIIH-----	ESQRGTDRIGYFPGGIVEVV
RKVRALMDYEA	-----AEDNELTFKAGEIITVLDDSS-----	DPNWWKGETH-----	QGIGLFPSNFVTAD
RKVRALMDYEA	-----VEDNELTFKHGEIITVLDDSS-----	DANWKKGENH-----	RGIGLFPSDFVTTN
TYVQALMDYEDP	-----QEDGELGFRRGDFIHMNDNS-----	DPNWWKGACH-----	GQTGMFPRNYVTPV
TYGVALMDYFOA	-----LEPNELDFEVGDKIRILATL-----	EDGWLEGSLLK-----	GRTGIFPRYFVKLC
PCCRALMDYEP	-----ENEGELGFKEGDIITLTNQI-----	DENWYEGMLH-----	GHSGFPPINYEVL
PCCRGLMDYEP	-----ENQDELGFKEGDIITLTNQI-----	DENWYEGMIH-----	GESGFPPINYEVI
PSCKALMDYEP	-----ENDGELGFHEGDVITLTNQI-----	DENWYEGMLD-----	QSGGFPLSYVEVL
RPARAKMDYKA	-----QTLKELPLQKGDIVYIYKQI-----	DQNWYEGEHH-----	GRVGIFPRTYIELL
KAARLKMDYFOA	-----QSPKELTLQKGDIVYIHKV-----	DKNWLEGEHH-----	GRLGIFPANYVEVL
LPAKALMDYKA	-----QTSKELSFKKGDTVYIILRKI-----	DQNWYEGEHH-----	GRVGIFPISYVEKL
PKYVGLMDYKS	-----RTDELSFRAGDVVFHVARKE-----	EQWWATLLD-----	EAGGAVAQGYVPHNYLAER
LKMOVLMDEFA	-----RNPRELTVVQGEKLEVLDS-----	KRWLVKNE-----	AGRSYIPSNILEPL
PCAKALMNEG	-----KEPGELKFSKGDIIILRRQV-----	DENWYHGEVN-----	GIHGFFPTNFVQII
SVVRAIMDFCP	-----SVSEELPLFVGDIIEVLAVV-----	DEFWLLGKKE-----	DVTGQFPSSFVEIV
RWARALMDYEA	-----LEDDELGFHSGEVVEVLDS-----	NPSWWTGRLLH-----	NKLGLFPANYVAPM
EYVRALMDYNG	-----NDEDELPPFKKGDILIRIDKP-----	EEQWNAEDS-----	EGKRGMI PVPYVEKY
EYVRTLMDYPG	-----NDABELPPFKKGEILVIEKP-----	EEQWWSARNK-----	DGRVGMIPVPYVEKL
IEAIAKMDYVG	-----RTARELSFKKGASLLLYQRA-----	SDDWWEGRHN-----	GIDGLIPHQYIVVQ
IEAIAKMDYVG	-----RSARELSFKKGASLLLYHRA-----	SEDWWEGRHN-----	GIDGLIPHQYIVVQ
IEAIAKMDYMG	-----RSPRELSPFKKGASLLLYHRA-----	SEDWWEGRHN-----	CVDGLIPHQYIVVQ
CQVIAMMDYAA	-----NNEDELSPFKGQILNVMNKD-----	DPDWQGEIN-----	GVTGLFSPSNYVKMT
CQVIAMMDYAA	-----NNEDELSPFKGQILNVMNKD-----	DPDWQGEIN-----	GVTGLFSPSNYVKMT
RTVKALMDYKA	-----KRSDELSPFCRGALIHNVSK-----	PGGWKGDYD-----	TRIQQYFSPSNYVEDI
CAVKALMDYKA	-----QREDELTFIKSAI IQNVEKQ-----	EGGWWRGDYD-----	GKKQLWFPSPNYVEEM
LCARALMDYQA	-----ADDTDELSPDPENLITGIEVI-----	DEGWWRGYGP-----	DGHFGMFPANYVELI
EKVVALMDYTK	-----DKEDELSPFQEGAI IYVIKKN-----	DDGWYEGVMN-----	GVTGLFPNGYVESI
EKVVALMDYTK	-----DKDDELSPFMEGAI IYVIKKN-----	DDGWYEGVCN-----	RVTGLFPNGYVESI
GLWALMDYEA	-----RGEDELSPLRGQLEVEVLSQD-----	AAVSGDEGWAGQVQ-----	RRLGIFPANYVAPC

Predicted protein
 Vav1 #2
 HEFL
 PACSIN1
 PACSIN2
 BPAG1
 MYOSIN I-f
 Cortactin/EMS1
 LckBP1 / HS1
 FLJ00204 #1
 Sorting nexin 9
 Sorting nexin 18
 KIAA0318 #3
 Homology to EEN-B1
 CIN85 #1
 CMS #1
 Frk/Rak
 p130Cas
 HEF1/CasL
 Efs/Sin
 Ponsin #3
 ArgBP2a #3
 Nebulette
 Lasp-1
 Nebulin
 Peroxin-13
 Intersectin 1L #1
 Intersectin 1 #1
 Intersectin 2 #1
 Mlk3
 Nostrin
 Amphiphysin II
 Amphiphysin
 CMS #2
 CD2BP1
 Mlk2
 PACSIN3
 P85A
 P85B
 Predicted protein
 MYOSIN I-C
 Btk
 Itk
 Eps8R2
 KIAA0318 #2
 Nck1 #1
 Nck2 #1
 Grap #1
 CASKIN #2
 STAM1
 STAM2
 Grb2 #2
 KIAA1010 #4
 EEN-B1/SH3GL2
 EEN-B2/SH3GL3
 EEN/SH3GL1
 Ponsin #1
 Vinexin #1
 ArgBP2a #1
 Brk
 Eps8R3
 POSH #1
 KIAA1010 #1
 GADS/Grp2 #2
 Crk #1
 CrkL #1
 SRGAP2
 SRGAP1
 SRGAP3
 Intersectin 2 #5
 Intersectin 2 #5
 PLC-gamma-2
 PLC-gamma-1
 HIP-55
 AblBP3
 Abl-1
 Mlk4

EAHRVLEGGVP-----ETKEELQVMPGNIVFVLKKG-----NDNWATVMFN-----GQKGLVPCNYLEPV

p67-phox #1